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10/535,312	06/05/2006	Sung Youb Jung	Q115522	5682

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EXAMINER	
BRISTOL, LYNN ANNE	

ART UNIT	PAPER NUMBER
1643	

NOTIFICATION DATE	DELIVERY MODE
10/15/2010	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

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Office Action Summary	Application No.	Applicant(s)	
	10/535,312	JUNG ET AL.	
	Examiner	Art Unit	
	LYNN BRISTOL	1643	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *24 August 2010*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-6, 8, 9, 11-13, 15 and 16* is/are pending in the application.

4a) Of the above claim(s) *15* is/are withdrawn from consideration.

5)☒ Claim(s) *13* is/are allowed.

6)☒ Claim(s) *1-6,8,9,11,12 and 16* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Continued Examination Under 37 CFR 1.114

1. A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 8/24/10 has been entered.

2. Claims 1-6, 8, 9, 11-13, 15 and 16 are all the pending claims for this application.

3. Claims 1-3 and 13 were amended in the Response of 8/24/10.

4. Claim 15 is withdrawn from examination.

5. Claims 1-6, 8, 9, 11-13 and 16 are all the pending claims under examination.

6. This Office Action contains new grounds for rejection.

Rejections Withdrawn

Claim Rejections - 35 USC § 112, first paragraph

Written Description

7. The rejection of Claims 1-6, 8, 9, 11-13 and 16 under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement because the claims are drawn to a negative proviso (e.g., method where an immunoglobulin constant region is… “not secreted into the medium”) unsupported by the specification is withdrawn.

Applicants have amended the generic claims to identify that it is the cytoplasm of the E. coli wherein the soluble Fc domain is expressed.

Rejections Maintained

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

The factual inquiries set forth in *Graham* **v.** *John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

8. The rejection of Claims 1-6, 8 and 16 under 35 U.S.C. 103(a) as being unpatentable over Kitai et al. (Appl. Microbiol. Biotechnol 28(1):52-56 (Mar. 1988); cited in the IDS of 10/9/09) in view of Simmons et al. (Nat. Biotech. 14:629-634 (1996)) and further in view of Sytkowski et al. (WO 99/02709; published 1/21/99); cited in the IDS of 10/9/09) is maintained.

The meaning of the term "processed" in amended Claim 1 is unclear because it does not limit the signal sequence being cleaved from the Fc protein nor exclude any physiological event occurring to the signal sequence, e.g., glycosylation.

For purposes of review, the rejection was set forth in the Office Action of 12/4/09 as follows:

"Claims 1-6, 8 and 16 are interpreted as being drawn to a method for producing a Ig Fc in the cytoplasm or secreted from an E coli having been transfected with a nucleotide encoding the STII signal sequence and the Ig Fc domain without a variable domain (Claim 1), where the Ig Fc region is from IgG, IgA, IgM, IgE or IgD, (Claim 2) or for the subtypes IgG1, IgG2, IgG3 and IgG4 (Claim 3), or IgG4 (Claim 4), where the Fc of Claim 4 is aglycosylated (Claim 5), and Fc comprises a portion of a hinge (Claim 6 and 16), and where the Fc is from a heavy or light chain (Claim 8).

It would have been prima facie obvious to have produced the instant claimed method for producing soluble Ig Fc domains from an E. coli in view of Kitai, Simmons and Sytkowski.

Kitai discloses a penicillinase signal peptide and hIgG-Fc were fused through the one additional amino acid, Ser. This hybrid protein was translocated "across the inner membrane, correctly processed between Ala and Set, and excreted into the culture medium in the dimeric form. These results indicate that this penicillinase signal peptide works efficiently, even when a foreign protein is fused. Kitai discloses plasmid pEAP8 was an excretion vector in *E. coli* transformants (Kate e tal. 1987) and containing the DNA region needed for the extracellular production in E. coli, that is KII gene of pMB9, Ex promoter.and penicillinase promoter-signal-peptide. Kitai does not teach using the heat-stable enterotoxin signal peptide or the constant regions from IgA, IgM, IgE or IgD, or for the subtypes IgG1, IgG2, IgG3 and IgG4 whereas do Simmons and Sytkowski.

Simmons discloses examples of three heat stable enterotoxin (STII) signal sequence derivatives differing only in the TIR and maintaining the wildtype amino acid sequence (Table 1, variants 1, 4, 6) which improved the secretion of a sample of heterologous proteins over wildtype STII constructs in *E coli* transformants. Simmons compared expression of a heterologous gene of interest inserted downstream of the phoA promoter, trp Shine-Delgarno and an STII signal sequence possessing a different relative TIR strength. Simmons teaches the optimal level of protein synthesis needs to be determined empirically for every heterologous protein and the panel of vectors with differing translational strengths provides a means by which to readily adjust this factor. Simmons appreciates producing heterologous proteins using the STII signal sequence but does not suggest the heterologous protein is the constant regions from IgA, IgM, IgE or IgD, or for the subtypes IgG1, IgG2, IgG3 and IgG4 whereas does Sytkowski. The IgG of Kitai would also be considered a heterologous protein with respect to the E. coli host expression system.

Sytkowski teaches cloning Fc domains from IgGl, IgG2, IgG2a, IgG3, IgG4, IgM, IgA, IgD or IgE of the heavy or light chain, where the Ig constant region comprises immunoglobulin hinge region, CH2 domain and CH3 domain or CL1 domain, respectively. Sytkowski teaches the entire immunoglobulin heavy chain constant region (CHI-hinge-CH2-CH3) or alternatively, the immunoglobulin constant region can comprise all, or a portion of the hinge region, the CH2 domain and the CH3 domain. The immunoglobulin constant region can also comprise the CL1 domain of an immunoglobulin light chain. Finally Simmons teaches a fusion protein may comprise a signal or targeting sequence (p. 5). The proteins of Sytkowski use the cloned Fc domains to create a fusion protein with cloned EPO, however, it is the examiner's position that the EPO portion is not important or essential and can be removed from the Sytkowski art reference method in setting forth this obviousness rejection, see Eisai Co. v. Dr. Reddy's Laboratories, 533 F.3d 1353, 1358 (Fed. Cir. 2008) (noting in regard to obviousness, that the record provided no reason to start with a lead compound and then drop the feature of the lead compound that leads to its advantageous properties) (cited at page 3 of the Reply Br.). The ordinary artisan would not have considered the EPO portion an essential element, such that its removal would render the method of Sytkowski inoperable. This is supported by the implied statements of Sytkowski that the Ig constant domain alone binds the Fc receptor or can have ADCC or ACC activity or extends the half-life of a molecule to which it is attached (p. 15), and thus to produce an isolated Fc would have advantages for other uses other than fusing it to EPO.

The ordinary artisan would have been motivated and reasonably assured of success in having produced the instant claimed method in view of Kitai, Simmons and Sytkowski. The references alone address the expression of cloned proteins in E. coli systems where Kitai and Simmons use different but otherwise interchangeable signal sequences and replacing the penicillinase sequence with the STII sequence of Simmons would seemingly improve

the product outcome in E. coli cytosol as well as secreted proteins. To have considered expressing an Fc protein was contemplated and reduced to practice by Kitai and therefore obvious, and further where Simmons appreciates using the STII to express many different heterologous proteins in E. coli, where contiguous or portions of Fc domains including portions of the hinge from different antibody isotypes and isoforms were contemplated by Sytkowski in a fusion protein format. The ordinary artisan would have appreciated that human proteins expressed in E. coli would not be glycosylated, so that an Fc from IgG4 would have been aglycosylated using the claimed method. The ordinary artisan would have been assured of success because the level of skill and technology and the reagents for producing isolated Fc's was already reduced to practice as set forth in the three references where in order to obtain an abundance of purified Fc proteins absent further manipulation than fusing the Fc to an STII sequence, the ordinary artisan could have predicted a reasonably achievable outcome."

The rejection was maintained in the Office Action of 2/25/10 as follows:

"Applicants allegations on pp. 7-9 of the Response of 2/4/10 and the amendment of Claim 1 to recite that the "the immunoglobulin constant region is expressed in the cytoplasm in a water soluble form and is not secreted into the medium" has been considered and is not found persuasive. Applicants allege the amended claims to introduce the new limitation obviate the rejection because neither Kitai alone or in combination teaches a method to produce a Fc region into a cytoplasm of a host transformant.

Response to Arguments

It is the examiner's opinion that the method would still have been obvious over Kitai, Simmons and Sytkowski despite the introduction of the new limitation.

Kitai teaches expression promoting plasmids for use in E. coli.

Simmons teaches that the optimal level of protein synthesis needs to be determined empirically for every heterologous protein and the panel of vectors with differing translational strengths provides a means by which to readily adjust this factor. Simmons teaches "the efficient transport of many heterologous proteins across a membrane is dependent upon the coupling of translation with translocation. The optimization of translational levels may represent the optimization of this coupling". Thus a reasonable conclusion to be drawn from Simmons is that absent efficient translation, enough of the heterologous protein would not be expected to be secreted into the medium and would otherwise be retained in the cytoplasm or periplasmic space. Simmons recognizes that the extent to which any heterologous protein is secreted depends on the particular construct, and Simmons teaches examples of constructs that are poor secreters. Thus the references teach examples of secreting and non-secreting constructs (see Figure 1 and Table 1) and the latter of which are encompassed by the instant claimed method.

Finally, in response to applicant's arguments against the Kitai reference individually, one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.,* 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986)."

Applicants allegations on p. 8 and p. 9 of the Response of 8/24/10 have been considered and are not found persuasive.

a) Applicants allege "none of the cited references teach the limitations "overexpress the immunoglobulin constant region in the cytoplasm of the transformant, wherein the signal sequence of the overexpressed immnunoglobulin constant region is processed" and "expressed in the cytoplasm in soluble form" of currently amended claim 1."

Response to Arguments

Applicants have not addressed the technical feature taught in Simmons that the method involves "processing of the precursor." Simmons also teaches on p. 629, Col. 1 that cytoplasmic or "intracellular expression is the more common technique used for high level production of proteins". Thus, the references combined teach all of the elements of the instant claims, namely, expressing "processed" proteins in a cytoplasm of an E. coli expression system.

b) Applicants allege "the combination of the STII signal sequence (among numerous leading sequences) and an Fc region (among numerous target proteins) is not obvious. In particular, such is true, when considering the facts that expression efficiency of the target proteins in a fusion protein varies greatly from one protein to another protein. Furthermore, it is more difficult and unpredictable to produce a target protein in a soluble form in the cytoplasm of E. coli."

Response to Arguments

Under 2144.07 Art Recognized Suitability for an Intended Purpose

"The selection of a known material based on its suitability for its intended use supported a prima facie obviousness determination in Sinclair & Carroll Co. v. Interchemical Corp., 325 U.S. 327, 65 USPQ 297 (1945)."

Applicants have not explained with technical or legal reasons why using an E. coli STII signal sequence in an E. coli expression based system much less where the fusion protein is a truncated antibody molecule, i.e., Fc domain, would not be expected to work.

Finally, and pursuant to MPEP 2144.03, "ordinarily there must be some form of evidence in the record to support an assertion of common knowledge." Here, Applicants assert on the record that it is common knowledge that 1) efficiency of the target proteins in a fusion protein varies greatly from one protein to another protein; and 2) it is more difficult and unpredictable to produce a target protein in a soluble form in the cytoplasm of E. coli.

The rejection is maintained.

9. The rejection of Claims 1, 9 and 12 under 35 U.S.C. 103(a) as being unpatentable over Kitai et al. (Appl. Microbiol. Biotechnol 28(1):52-56 (Mar. 1988); cited in the IDS of 10/9/09) in view of Simmons et al. (Nat. Biotech. 14:629-634 (1996)) and further in view of Sytkowski et al. (WO 99/02709; published 1/21/99); cited in the IDS of 10/9/09) as applied to claim 1 above, and further in view of Lilly (US 20040053370; filed 5/29/03) is maintained.

For purposes of review, the rejection was set forth in the Office Action of 12/4/09 as follows:

"The interpretation of Claim 1 is discussed above under section 10. Claims 9 and 12 are drawn to the Fc isotype for IgG4 of SEQ ID NO:29.

Lilly teaches an Fc sequence having 100% identity to SEQ ID NO 29 of Claims 9 and 12 (see attached sequence search alignment) and used to construct fusion proteins. Lilly teaches "[0238] Based on these studies, Fc regions can be modified at the catabolic site to optimize the half-life of the fusion proteins. It is preferable that the Fc region…be derived from an IgG1 or an IgG4 Fc region…and even more preferable that the Fc region be IgG4or derived from IgG4. Preferably the IgG Fc region contains both the CH2 and CH3 regions including the hinge region. Thus in view of Kitai, Simmons and Sytkowski, the ordinary artisan would have found motivation use the IgG4 Fc of Lilly in the construct of Kitai in view of Simmons and Sytkowski where according to Lilly the IgG4 Fc is preferable.

The rejection was maintained in the Office Action of 2/25/10 as follows:

"Applicants allegations on pp. 7-9 of the Response of 2/4/10 and the amendment of Claim 1 to recite that the "the immunoglobulin constant region is expressed in the cytoplasm in a water soluble form and is not secreted into

the medium" has been considered and is not found persuasive. Applicants allege the amended claims to introduce the new limitation obviate the rejection because neither Kitai alone or in combination teaches a method to produce a Fc region into a cytoplasm of a host transformant.

Response to Arguments

It is the examiner's opinion that the method would still have been obvious over Kitai, Simmons, Sytkowski and Lilly despite the introduction of the new limitation.

Kitai teaches expression promoting plasmids for use in E. coli. Simmons teaches that the optimal level of protein synthesis needs to be determined empirically for every heterologous protein and the panel of vectors with differing translational strengths provides a means by which to readily adjust this factor. Simmons teaches "the efficient transport of many heterologous proteins across a membrane is dependent upon the coupling of translation with translocation. The optimization of translational levels may represent the optimization of this coupling". Thus a reasonable conclusion to be drawn from Simmons is that absent efficient translation, enough of the heterologous protein would not be expected to be secreted into the medium and would otherwise be retained in the cytoplasm or periplasmic space. Simmons recognizes that the extent to which any heterologous protein is secreted depends on the particular construct, and Simmons teaches examples of constructs that are poor secreters. Thus the reference teach examples of secreting and non-secreting constructs (see Figure 1 and Table 1) and the latter of which are encompassed by the instant claimed method.

Finally, in response to applicant's arguments against the Kitai reference individually, one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.,* 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986).

Applicants allegations on p. 8 and p. 9 of the Response of 8/24/10 have been considered and are not found persuasive. See the summary of Applicants allegations and the rebuttal of the examiner set forth under section 8 above.

10. The rejection of Claims 1 and 11 under 35 U.S.C. 103(a) as being unpatentable over Kitai et al. (Appl. Microbiol. Biotechnol 28(1):52-56 (Mar. 1988); cited in the IDS of 10/9/09) in view of Simmons et al. (Nat. Biotech. 14:629-634 (1996)) and further in view of Sytkowski et al. (WO 99/02709; published 1/21/99); cited in the IDS of 10/9/09) as applied to claim 1 above, and further in view of Kwon et al. (WO200015661; published 3/23/00) is maintained.

For purposes of review, the rejection was set forth in the Office Action of 12/4/09 as follows:

"The interpretation of Claim 1 is discussed above under section 10. Claim 11 is drawn to the heat stable enterotxin signal sequence of SEQ ID NO: 36, 37, 38, 40, 41, 42, 43, 44, 45, or 46.

Kwon teaches heat stable enterotoxin II signal sequence having 100% identity to SEQ ID NO: 36, 37, 38, 39, 40, 41, 42, 43, 44, 45 and 46 of Claim 11 (see attached sequence search alignments) and used to construct fusion proteins. Kwon teaches the modified signal sequences enhance the efficiency of peptide secretion from the E. coli cells, and the modified signal peptides may be used according to standard recombinant DNA methodolgies to direct the secretion of peptides from microorganisms (Abstract). Thus in view of Kitai, Simmons and Sytkowski, the ordinary artisan would have found motivation to use the modified signal peptide sequences of Kwon in the construct of Kitai in view of Simmons and Sytkowski where the ordinary artisan would be reasonably assured that the signal peptides would have enhanced the secretion of Fc."

The rejection was maintained in the Office Action of 2/25/10 as follows:

"Applicants allegations on pp. 7-9 of the Response of 2/4/10 and the amendment of Claim 1 to recite that the "the immunoglobulin constant region is expressed in the cytoplasm in a water soluble form and is not secreted into the medium" has been considered and is not found persuasive. Applicants allege the amended claims to introduce the new limitation obviate the rejection because neither Kitai alone or in combination teaches a method to produce a Fc region into a cytoplasm of a host transformant.

Response to Arguments

It is the examiner's opinion that the method would still have been obvious over Kitai, Simmons, Sytkowski and Kwon despite the introduction of the new limitation.

Kitai teaches expression promoting plasmids for use in E. coli.

Simmons teaches that the optimal level of protein synthesis needs to be determined empirically for every heterologous protein and the panel of vectors with differing translational strengths provides a means by which to readily adjust this factor. Simmons teaches "the efficient transport of many heterologous proteins across a membrane is dependent upon the coupling of translation with translocation. The optimization of translational levels may represent the optimization of this coupling". Thus a reasonable conclusion to be drawn from Simmons is that absent efficient translation, enough of the heterologous protein would not be expected to be secreted into the medium and would otherwise be retained in the cytoplasm or periplasmic space. Simmons recognizes that the extent to which any heterologous protein is secreted depends on the particular construct, and Simmons teaches examples of constructs that are poor secreters. Thus the reference teaches examples of secreting and non-secreting constructs (see Figure 1 and Table 1) and the latter of which are encompassed by the instant claimed method.

Finally, in response to applicant's arguments against the Kitai reference individually, one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.,* 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986)."

Applicants allegations on p. 8 and p. 9 of the Response of 8/24/10 have been considered and are not found persuasive. See the summary of Applicants allegations and the rebuttal of the examiner set forth under section 8 above.

11. The rejection of Claims 1-6, 8 and 16 under 35 U.S.C. 103(a) as being unpatentable over Andrews et al. (Gene 182:101-109 (1996); cited in the IDS of 1/5/10) in view of Simmons et al. (Nat. Biotech. 14:629-634 (1996); cited in the PTO 892 form of

12/4/09) and further in view of Sytkowski et al. (WO 99/02709; published 1/21/99); cited in the IDS of 10/9/09) is maintained.

The rejection was set forth in the Office Action of 2/25/10 as follows:

"Claims 1-6, 8 and 16 are interpreted as being drawn to a method for producing a Ig Fc that is soluble and is not secreted into the medium from an E coli having been transfected with a nucleotide encoding the STII signal sequence and the Ig Fc domain without a variable domain (Claim 1), where the Ig Fc region is from IgG, IgA, IgM, IgE or IgD, (Claim 2) or for the subtypes IgG1, IgG2, IgG3 and IgG4 (Claim 3), or IgG4 (Claim 4), where the Fc of Claim 4 is aglycosylated (Claim 5), and Fc comprises a portion of a hinge (Claim 6 and 16), and where the Fc is from a heavy or light chain (Claim 8). The negative proviso is interpreted as meaning that the protein is secreted in the cytoplasm.

It would have been prima facie obvious to have produced the instant claimed method for producing soluble Ig Fc domains from an E. coli in view of Andrews, Simmons and Sytkowski.

Andrews discloses the expression of a polypeptide in E. coli, using the STII signal sequence leading to cytoplasmic expression (pp. 104-105):

"The STII leader is expected to facilitate transport of V135.3 to the periplasm and thus expedite the facile purification of VI35.3 following osmotic shock. In addi\- tion, the potential folding of V[35.3 which contains two cysteine residues might be expedited in the more oxidizing environment of the periplasmic space, relative to the cytosol. However, we found that V135.3 expressed from all vectors was localized in the cytosol despite the presence of the STII leader sequence in the constructs. Furthermore, amino acid sequence analysis showed that even without translocation of V135.3 to the periplasm, the leader peptide had been cleaved off correctly.",

and on p. 106, Col.2:

"Irrespective of its localization, a statistical model based on amino acid composition (Wilkinson and Harrison, 1991) predicts a 95% probability of V135.3 being insoluble when expressed in E. coli."

Andrews does not teach using the heat-stable enterotoxin signal peptide resulting in a soluble protein or the constant regions from IgA, IgM, IgE or IgD, or for the subtypes IgG1, IgG2, IgG3 and IgG4 whereas do Simmons and Sytkowski.

Simmons discloses examples of three heat stable enterotoxin (STII) signal sequence derivatives differing only in the TIR and maintaining the wildtype amino acid sequence (Table 1, variants 1, 4, 6) which improved the secretion of a sample of heterologous proteins over wildtype STII constructs in *E coli* transformants. Simmons compared expression of a heterologous gene of interest inserted downstream of the phoA promoter, trp Shine-Delgarno and an STII signal sequence possessing a different relative TIR strength. Simmons teaches the optimal level of protein synthesis needs to be determined empirically for every heterologous protein and the panel of vectors with differing translational strengths provides a means by which to readily adjust this factor.

Simmons teaches "the efficient transport of many heterologous proteins across a membrane is dependent upon the coupling of translation with translocation. The optimization of translational levels may represent the optimization of this coupling". Thus a reasonable conclusion to be drawn from Simmons is that absent efficient translation, enough of the heterologous protein would not be expected to be secreted into the medium and would otherwise be retained in the cytoplasm or periplasmic space. Simmons recognizes that the extent to which any heterologous protein is secreted depends on the particular construct, and Simmons teaches examples of constructs that are poor secreters. Thus the reference teaches examples of secreting and non-secreting constructs (see Figure 1 and Table 1) and the latter of which are encompassed by the instant claimed method.

Simmons appreciates producing heterologous proteins using the STII signal sequence but does not suggest the heterologous protein is the constant regions from IgA, IgM, IgE or IgD, or for the subtypes IgG1, IgG2, IgG3 and IgG4 whereas does Sytkowski.

Sytkowski teaches cloning Fc domains from IgGl, IgG2, IgG2a, IgG3, IgG4, IgM, IgA, IgD or IgE of the heavy or light chain, where the Ig constant region comprises immunoglobulin hinge region, CH2 domain and CH3 domain or CL1 domain, respectively. Sytkowski teaches the entire immunoglobulin heavy chain constant region (CHI-hinge-CH2-CH3) or alternatively, the immunoglobulin constant region can comprise all, or a portion of the hinge region, the CH2 domain and the CH3 domain. The immunoglobulin constant region can also comprise the CL1 domain of an immunoglobulin light chain. Finally Simmons teaches a fusion protein may comprise a signal or targeting sequence (p. 5). The proteins of Sytkowski use

the cloned Fc domains to create a fusion protein with cloned EPO, however, it is the examiner's position that the EPO portion is not important or essential and can be removed from the Sytkowski art reference method in setting forth this obviousness rejection (see *Eisai Co. v. Dr. Reddy's Laboratories*, 533 F.3d 1353, 1358 (Fed. Cir. 2008) (noting in regard to obviousness, that the record provided no reason to start with a lead compound and then drop the feature of the lead compound that leads to its advantageous properties) (cited at page 3 of the Reply Br.). The ordinary artisan would not have considered the EPO portion an essential element, such that its removal would render the method of Sytkowski inoperable. This is supported by the implied statements of Sytkowski that the Ig constant domain alone binds the Fc receptor or can have ADCC or ACC activity or extends the half-life of a molecule to which it is attached (p. 15), and thus to produce an isolated Fc would have advantages for other uses other than fusing it to EPO.

The ordinary artisan would have been motivated and reasonably assured of success in having produced the instant claimed method in view of Andrews, Simmons and Sytkowski. The references alone address the expression of cloned proteins in E. coli systems where Andrews and Simmons use different but otherwise interchangeable STII signal sequences and replacing the Andrews sequence that does not produce soluble proteins with the STII sequence of Simmons would seemingly improve the product outcome in E. coli cytosol as well as secreted proteins, e.g., periplasm. To have considered expressing an heterologous protein was contemplated by Andrews (p. 108) and therefore obvious further where Simmons appreciates using the STII to express many different heterologous proteins in E. coli, and where contiguous or portions of Fc domains including portions of the hinge from different antibody isotypes and isoforms were contemplated by Sytkowski in a fusion protein format. The ordinary artisan would have appreciated that human proteins expressed in E. coli would not be glycosylated, so that an Fc from IgG4 would have been aglycosylated using the claimed method. The ordinary artisan would have been assured of success because the level of skill and technology and the reagents for producing isolated Fc's was already reduced to practice as set forth in the Sytkowski reference where in order to obtain an abundance of purified Fc proteins absent further manipulation than fusing the Fc to an STII sequence, the ordinary artisan could have predicted a reasonably achievable outcome."

Applicants allegations on p. 8 of the Response of 8/24/10 have been considered and are not found persuasive.

a) Applicants allege Andrew discloses that a target protein combined with the STII signal sequence is expressed in the cytoplasm. However, the target protein in Andrew is expressed in insoluble form (page 106, right column). Therefore, Andrew, either alone or in combination with Simmons, fails to teach all and every limitation of currently amended claim 1.

Response to Arguments

The text from "page 106, right column" of Andrews states the following:

"Irrespective of its localization, a statistical model based on amino acid composition (Wilkinson and Harrison, 1991) predicts a 95% probability of V135.3 being insoluble when expressed in E. coli."

Here the examiner submits that none of the claims recite a quantitative requirement regarding the amount of soluble Fc protein actually being accumulated in the cytoplasm. The claims require overexpression of the Fc protein but no limitation that the overexpressed Fc protein is an overexpressed soluble form. Finally, the statement of Andrews applies to the empirical prediction for the V135.3 protein and not the universe of heterologous proteins expressed in E. coli. Applicants are requested to identify the support and disclosure in Andrews that the predictive estimate for the V135.3 protein can be extrapolated to the Fc protein, otherwise, Applicants assertions are baseless and irrelevant.

The rejection is maintained.

12. The rejection of Claims 1, 9 and 12 under 35 U.S.C. 103(a) as being unpatentable over Andrews et al. (Gene 182:101-109 (1996); cited in the IDS of 1/5/10) in view of Simmons et al. (Nat. Biotech. 14:629-634 (1996); cited in the PTO 892 form of 12/4/09) and further in view of Sytkowski et al. (WO 99/02709; published 1/21/99); cited in the IDS of 10/9/09) as applied to claim 1 above, and further in view of Lilly (US 20040053370; filed 5/29/03); cited in the Office Action of 12/4/09) is maintained.

The rejection was set forth in the Office Action of 2/25/10 as follows:

"Claims 9 and 12 are drawn to the Fc isotype for IgG4 of SEQ ID NO:29.

Lilly teaches an Fc sequence having 100% identity to SEQ ID NO 29 of Claims 9 and 12 (see sequence search alignment in the PTO 892 form of 12/4/09) and used to construct fusion proteins. Lilly teaches "[0238] Based on these studies, Fc regions can be modified at the catabolic site to optimize the half-life of the fusion proteins. It is preferable that the Fc region...be derived from an IgG1 or an IgG4 Fc region...and even more preferable that the Fc region be IgG4or derived from IgG4. Preferably the IgG Fc region contains both the CH2 and CH3 regions including the hinge region. Thus in view of Andrews, Simmons and Sytkowski, the ordinary artisan would have found motivation use the IgG4 Fc of Lilly in the construct of Andrews in view of Simmons and Sytkowski where according to Lilly the IgG4 Fc is preferable."

Applicants allegations on p. 8 of the Response of 8/24/10 have been considered and are not found persuasive. See the summary of Applicants allegations and the rebuttal of the examiner set forth under section 11 above.

13. The rejection of Claims 1 and 11 under 35 U.S.C. 103(a) as being unpatentable over Andrews et al. (Gene 182:101-109 (1996); cited in the IDS of 1/5/10) in view of Simmons et al. (Nat. Biotech. 14:629-634 (1996)) and further in view of Sytkowski et al. (WO 99/02709; published 1/21/99); cited in the IDS of 10/9/09) as applied to claim 1 above, and further in view of Kwon et al. (WO200015661; published 3/23/00; cited in the PTO 892 form of 12/4/09) is maintained.

The rejection was set forth in the Office Action of 2/25/10 as follows:

"Claim 11 is drawn to the heat stable enterotxin signal sequence of SEQ ID NO: 36, 37, 38, 40, 41, 42, 43, 44, 45, or 46.

Kwon teaches heat stable enterotoxin II signal sequence having 100% identity to SEQ ID NO: 36, 37, 38, 39, 40, 41, 42, 43, 44, 45 and 46 of Claim 11 (see attached sequence search alignments) and used to construct fusion proteins. Kwon teaches the modified signal sequences enhance the efficiency of peptide secretion from the E. coli cells, and the modified signal peptides may be used according to standard recombinant DNA methodologies to direct the secretion of peptides from microorganisms (Abstract). Thus in view of Andrews, Simmons and Sytkowski, the ordinary artisan would have found motivation to use the modified signal peptide sequences of Kwon in the construct of Andrews in view of Simmons and Sytkowski where the ordinary artisan would be reasonably assured that the signal peptides would have enhanced the secretion of Fc."

Applicants allegations on p. 8 of the Response of 8/24/10 have been considered and are not found persuasive. See the summary of Applicants allegations and the rebuttal of the examiner set forth under section 11 above.

New Grounds for Rejection

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

14. Claims 1-6, 8, 9, 11, 12 and 16 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 1-6, 8, 9, 11, 12 and 16 are indefinite for the recitation "wherein the signal sequence of the overexpressed immnunoglobulin constant region is processed;" in Claim 1 because the meaning of the term processed is unclear. The specification does not provide a definition for the term and one skilled in the art would not know whether processing referred to any intracellular events relating to protein biochemistry such as glycosylation. The specification teaches on p. 47:

"As a result, the IgGl Fc protein was found to have an N-terminal sequence of Pro-Cys-Pro-Ala-Pro-Glu-Leu-Leu-Gly- Gly, the IgG4 Fc protein had an N-terminal sequence of Ser- Cys-Pro-Ala-Pro-Glu-Phe-Leu-Gly-Gly, and the IgG2 Fc protein had an N-terminal sequence of Pro-Cys-Pro-Ala-Pro- Pro-Val-Ala-Gly-Pro. As apparent from these results, the Fc fragments expressed by the E. coli transformants of the present invention were found to have an accurate N-terminal sequence. These results indicate that, when expressed in a form fused to a signal sequence, the Fc fragments are not secreted to the extracellular membrane or periplasmic space, are accurately processed in the signal sequence even upon overexpression and are present in a water-soluble form in the cytosol."

The specification does not teach that the Fc domain was cleaved from the signal sequence, only that upon sequencing, the N-terminal portion of the Fc domain comprised the above referenced sequences.

Conclusion

15. Claim 13 is in condition for allowance.

16. Any inquiry concerning this communication or earlier communications from the examiner should be directed to LYNN BRISTOL whose telephone number is (571)272-6883. The examiner can normally be reached on 8:00-4:30, Monday through Friday.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Misook Yu can be reached on 571-272-0839. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Lynn A. Bristol/
Primary Examiner, Art Unit 1643